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Researchers Bowled Over by Glutamate Transporter's Elegant

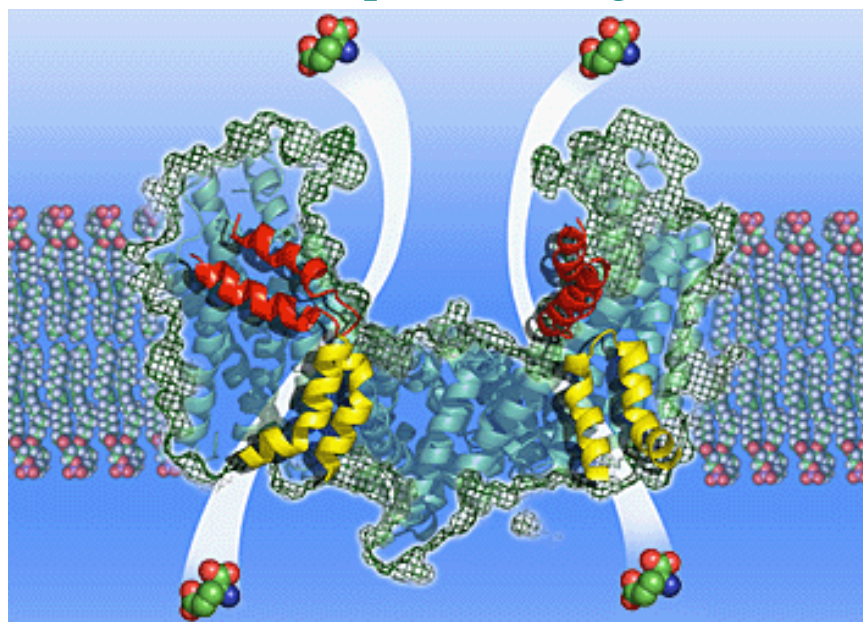


Image Title: The illustration depicts a high affinity glutamate transporter and shows a characteristic deep aqueous basin that reaches halfway across the membrane and two helical hairpins that cradle the glutamate binding site. Shown above is a slice through the center of the glutamate transporter from *Pyrococcus horikoshii*. - Olga Boudker/Laboratory of Eric Gouaux

A transporter protein that vacuums up the neurotransmitter glutamate has a structure radically different from any other membrane protein studied to date. Researchers are excited about the studies because they hope they will further illuminate the activity of glutamate transporters, proteins that shuttle the critical neurotransmitter between nerve cells.

The Howard Hughes Medical Institute (HHMI) researchers who determined the first-ever three-dimensional structure of such a neurotransmitter transporter found that the protein possesses a bowl shape that inserts deep into the cell membrane. The structure shows that the bowl contains protein segments that behave like flippers in a pinball machine to trap glutamate and retrieve it into the neuron.

"We had to do quite a bit of tinkering to get those crystals to diffract well, primarily because these proteins are extraordinarily hydrophobic, like grease balls, and they just don't tend to form nice lattices."

Glutamate-triggered neurons play a central role in learning and memory. Their dysfunction has been implicated in a wide range of disorders, including schizophrenia, depression and stroke, said HHMI investigator Eric Gouaux, who led the research team. Gouaux and co-lead authors Dinesh Yernool and Olga Boudker at Columbia University published their findings in the October 14, 2004, issue of the journal *Nature*.

"These transporters are absolutely essential for the cycle of signaling between neurons that is fundamental to brain function," Gouaux said.

"Neurotransmitter transport proteins are clinically important because, for example, they are the targets of the widely used anti-depressants called SSRIs, or selective serotonin reuptake inhibitors." Such drugs include Prozac, Celexa and Zoloft.

Gouaux said that basic understanding of the glutamate transporter could aid development of new drugs to treat a wide range of disorders. The transporter structure could also spur basic research because glutamate transporters are present in many organs, including the heart, kidney and intestine, although their function there is unknown.

Neurons in the brain trigger nerve impulses in their neighbors by launching bursts of neurotransmitter molecules across the synapse, which is the junction between neurons. After a neuron fires, it must go through a brief recovery period, during which it deploys transporter molecules to rapidly reuptake and recycle neurotransmitter molecules.

But until Gouaux and his colleagues determined the three-dimensional structure of the glutamate transporter, little was known about how such neurotransmitter transporters functioned, or even how they embedded themselves in the membrane of neurons.

"One of the most fascinating things about these proteins is that there were substantial ambiguities in the basic understanding of how they threaded across the membrane," said Gouaux. Furthermore, even though researchers had determined the amino acid sequence of the glutamate transporter protein, and performed biochemical experiments to aid in understanding its overall shape, major puzzles remained about the transporter's architecture and precise mechanism of action.

In their studies, Gouaux and his colleagues employed x-ray crystallography, a widely used analytical technique, to determine the transporter protein's structure. In this technique, x-rays are directed through crystals of a protein,

and its structure is deduced from the pattern of diffraction of the x-rays.

Producing crystals of the transporter protein presented considerable technical challenges, said Gouaux. "The job of these molecules is to move a substrate from one side of the membrane to the other," he said. "And to do this job, they have to be highly flexible. This complicates their crystallization because flexible, mobile, dynamic molecules do not produce good crystals." To make matters worse, such proteins are reluctant to form usable crystals because they contain hydrophobic, "water-hating" amino acids that enable them to sit in the cell's fatty membrane.

Thus, the researchers had to find a close analog of the human glutamate transporter protein that would adopt a more stable conformation, which would make it easier to crystallize. They found their match in a glutamate transporter from the bacterium *Pyrococcus horikoshii*, which has adapted to live in boiling undersea vents, and thus its proteins are less fragile.

Even then, the researchers had to induce selective mutations in the molecule to render it more amenable to analysis. "We had to do quite a bit of tinkering to get those crystals to diffract well, primarily because these proteins are extraordinarily hydrophobic, like grease balls, and they just don't tend to form nice lattices," said Gouaux.

The resulting glutamate transporter protein structure, said Gouaux, yielded extraordinary surprises. "The really fantastic element of this structure is that it has a bowl shape that bobs in the membrane and reaches halfway across it, exposed to the extracellular solution," he said. "A major question in the study of transporters has been how they move glutamate or other substrates across the membrane. And it's absolutely wonderful to see how this protein has accomplished it by carving out this bowl in the membrane. It's not as though there is some small channel that the glutamate molecule has to wiggle its way through before it gets halfway across the membrane bilayer. There's this giant bowl so it can just diffuse around and then find these binding sites."

The transporter's binding sites for glutamate represent another striking feature of the protein, said Gouaux. "The binding sites are flanked by helical hairpin structures that we've proposed act like trap doors, or flippers. When they open, they make the binding site accessible from one side of the membrane or the other." Each binding site has two types of such "flippers," said Gouaux.

"Crudely speaking, we think that these two flippers provide alternating access to the glutamate binding site that allows glutamate to move from the outside to the inside of the cell," he said.

The researchers' next goal is to understand in more detail how the *Pyrococcus* transporter functions. Gouaux and his colleagues are progressing to explore the structure of human transporters. He is optimistic that both types of studies will yield clinically useful insights into how drugs bind to and affect glutamate transporter proteins.